ALTERNATION OF GENERATIONS IN CERTAIN FLORIDEAE

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Cytological observations on *Polysiphonia* by YAMANOUCHI[1], *Griffithsia* by myself,[2] and *Delesseria* by SVEDELIUS,[3] render it probable that in these genera at least, and presumably in all Florideae in which tetraspores and sexual organs are regularly borne on separate individuals, there exists an alternation of sexual and asexual plants, the carpospores giving rise on germination to asexual, and the tetraspores to sexual individuals. For the past two summers at Wood's Hole I have been engaged in putting this matter to the proof by actual cultivation of the sporelings to maturity. This would seem to be a simple matter, but experience has shown me, as it has many others, that the range of physiological tolerance of these forms is extremely small. Temperature, light, and other less easily regulated factors may vary only within very narrow limits. All attempts to carry sporelings to maturity in the laboratory having failed, a plan was adopted similar to that employed by HOYT[4] in solving an analogous problem in Dictyota.

The method consisted in sowing spores on oyster shells, and transferring these to the open water after the sporelings had become firmly attached. The shells were soaked for at least 24 hours in fresh water and then scraped clean with a brush. Three small holes were made in each to facilitate its subsequent attachment. The shells so prepared were placed in deep glass vessels in filtered sea water. Fruiting algae of the desired species were then washed in running water for a few minutes, and placed in the vessels over

[1] YAMANOUCHI, S., The life history of *Polysiphonia*. BOT. GAZ. **42**:401–449. 1906.

[2] LEWIS, I. F., The life history of *Griffithsia Bornetiana*. Ann. Botany **23**:639–690. 1909.

[3] SVEDELIUS, N., Ueber den Generationswechsel bei *Delesseria sanguinea*. Svensk Botanisk Tidskrift **5**:260–324. 1911.

[4] HOYT, W. D., Alternation of generations and sexuality in *Dictyota dichotoma*. BOT. GAZ. **49**:55–57. 1910.

the shells. This was usually done in the afternoon, and the algae taken out the next morning. The spores of most species were shed in abundance, the resulting sporelings becoming attached almost immediately. At first the dishes were kept in the laboratory till the sporelings had become about 2 mm. long, and the shells were then transferred to the waters of Vineyard Sound. In such cases, however, the sudden change of environment exerted a deleterious effect on growth. For this reason, in all the later experiments the shells were transferred on the second day after the spores were shed. This was found to allow ample time for firm attachment of the sporelings. Each shell, before being "planted," was minutely inspected with a lens, and only those were used which showed a good uniform "stand" of sporelings without visible contamination from the spores of other species. Close inspection was especially necessary in the case of *Griffithsia* and various species of the Rhodomelaceae, on account of the frequency in these forms of vegetative multiplication from the broken off tips of the filaments. No attempt to thin out the sporelings was made.

Tarred cord, first soaked for some months in salt water to extract the soluble matter in the tar, was used for attaching the shells. A sort of ladder was made by having two parallel cords about 6 inches apart, to which shells were tied by cord running from the three holes mentioned. A ladder prepared in this way was either stretched horizontally between two supports, such as stakes firmly driven into the bottom or allowed to hang vertically from a pile. In the latter case the parallel cords were weighted at the bottom, to prevent the ladder from becoming tangled by the tidal currents. The latter method proved more satisfactory, as in this way, by having a series of shells bearing sporelings of one species, the optimum depth for the growth of that species could be readily determined. This was found to vary with the species employed, but in general it was about 2–3 feet below mean low water.

Plantations were made at Spindle Ledge, on the piles of the Government Wharf at Little Harbor, and on the piles at the end of the Fay Wharf. The best results were obtained at the last mentioned locality. The water here is very deep, the outermost

piles standing in the edge of the main channel. The rapid flow of the tides, and perhaps other factors, seem to prevent the attachment of spores, so that the algal flora at this point is quite scanty. This was found to be of importance for the reason that shells placed here showed almost no contamination from unknown sources, while at the other localities foreign spores settled and grew so abundantly and luxuriantly that the planted spores were overcrowded. Furthermore, at Spindle Ledge a considerable mass of drift, consisting for the most part of tangled mats of *Ectocarpus*, caught on the cords and prevented the development of the algae sown. In all, about 125 shells were planted.

The plantations were all made in July. The shells were left in the water 21–45 days. It was found, however, that in those species in which definite results were obtained, little if any growth took place after August 15. On all shells taken up as late as September 1, the algae were found to be disintegrating. After being collected, each shell was examined with the greatest care. Every visible growth, animal or plant, was scraped off and minutely inspected under the compound microscope. In this way possibility of error of observation was eliminated as far as could be done. In the course of the microscopic examinations, many observations were recorded as to the rate of growth of various species, the relative abundance of spores at different localities, and other matters not included within the scope of the present paper.

The following species were used: *Spermothamnion Turneri* Aresch., *Callithamnion Baileyi* Harv., *Griffithsia Bornetiana* Farlow, *Ceramium rubrum* Ag., *Ceramium fastigiatum* Harv., *Cystoclonium purpurascens* Kütz., *Chondrus crispus* (L.) Stack., *Lomentaria uncinata* Menegh., *Champia parvula* (Ag.) Harv., *Agardhiella tenera* (J. Ag.) Schmitz, *Grinnellia americana* Harv., *Gracilaria multipartita* J. Ag., *Chondria tenuissima* (Ag.) Harv., *C. dasyphila* (Ag.) Harv., *Polysiphonia fibrillosa* Grev., *P. violacea* Grev., *Dasya elegans* Ag. (17 species). Of these, the majority proved to be unsuitable, definitive results being obtained only with *Polysiphonia violacea, Griffithsia,* and *Dasya.* The failure to obtain results with some was due to lack of proper environment, since no shells were planted in deep water where such forms as *Gracilaria, Spermotham-*

nion, and *Cystoclonium* are found. In other cases, as *Chondrus* and *Agardhiella*, growth of the sporelings was so slow that they became covered and killed by the growth of exotic spores. In still others, the sporelings failed to become established, even the short time in which they were under laboratory conditions being sufficient to cause them to cease growing. Strange to say, some of these, as *Ceramium rubrum* and *Chondria tenuissima*, seem to be quite hardy, and are abundant and luxuriant in situations similar to those in which the shells were planted. Still others, including *Champia* and *Lomentaria*, were early discarded because of the frequency with which these species establish themselves on any suitable solid support.

Record of experiments

I. *Agardhiella tenera.*—Tetraspores and carpospores sown July 18, 1910, transferred to Spindle Ledge July 19. Shells collected August 10, and found to be much overgrown with foreign algae. Search revealed hundreds of small sterile *Agardhiella* plants, of which the average length was 0.5 mm. Some were of good color and apparently vigorous, but the great majority had ceased growing and were beginning to become discolored. The most interesting point about this culture was the fact that the sporelings from carpospores and tetraspores were of exactly the same size and conformation.

II. *Grinnellia americana.*—Treatment like that of *Agardhiella.* Hundreds of sterile specimens were found, 3–4 mm. long, vigorous and of good color. The fact that the amount of growth in 24 days at the height of the growing season was so slight would seem to indicate that this species, like the preceding one, is biennial, the sporelings of one summer reaching maturity the next year.

III. *Polysiphonia violacea.*—Tetraspores and carpospores sown July 18, 1911, transferred to piles at end of Fay Wharf July 20, collected August 14. The shells sown with tetraspores were accidentally destroyed before being collected. On examination, the shells sown with carpospores were found to be pretty thickly overgrown with *Polysiphonia variegata*, mostly sexual. Scattered among these plants, however, were 29 individuals of *P. violacea*, varying in length from 1.0 to 3.1 cm. The color of all was darker

than usual, and the plants seemed to be stunted. The main branches ended much more abruptly than is common in this species, and the development of hairs was sparse. The apices, however, were normal, and the plants still growing. All the smaller individuals, 23 in number, were found to be sterile, but 6, comprising the largest specimens, bore tetraspores normally and abundantly. On neighboring shells, which were examined very carefully as controls, no *P. violacea* was found. The only contaminating species were *P. variegata* in great numbers, *Champia parvula* (averaging 3 individuals to each shell), and *Dasya elegans* (2 per shell). It may be remarked parenthetically that *P. violacea* and *P. variegata* are species that are quite distinct, and easily and surely recognizable.

In this experiment, carpospores were found to produce tetrasporic plants, and no sexual individuals.

IV. *Griffithsia Bornetiana.*—Carpospores sown July 18, 1910, transferred to Spindle Ledge July 19, shells collected August 12. The stand of *Griffithsia* was found to be unusually good, the shells resembling miniature lawns on which *Griffithsia* was the grass. The individuals were so closely appressed and interwoven at the base that it was impossible to ascertain the exact number, which must, however, have reached into the hundreds. The largest were about 2 cm. long, a size at which sexual individuals fruit fairly abundantly, but all were sterile.

Tetraspores sown July 2, transferred to Spindle Ledge July 18, 1910, shells collected August 10. The maximum length attained was 1.5 cm., and the average 0.75 cm. Numerous very small sterile individuals were found. Of those specimens which had attained a length of 1.0 cm., 23 showed developing sexual organs, and 7 were sterile. Of the sexual plants, 12 were male and 11 female. Neighboring shells used as controls were destitute of *Griffithsia*. The results of this experiment were confirmed by cultures made in 1911. Tetraspores from a single plant were sown July 15, transferred to piles at Little Harbor July 18, shells collected August 14. The largest individuals were stocky, bushy plants 3 cm. long, and all were well developed, there being little crowding with the resulting production of dwarfed specimens. From one shell 45 individuals were obtained, of which 8 were sterile,

20 male, and 17 female. In the female plants ripe carpospores were being produced at the time of collection. Neighboring shells, as before, showed no *Griffithsia*. The contaminating species were *Champia parvula* (5 per shell), *Lomentaria uncinata* (4), *Grinnellia americana* (1), and *Polysiphonia variegata* (1).

A very interesting feature of this culture was the occurrence of two large apparently hermaphroditic individuals. In each case, close inspection revealed the fact that the apparently single individual was really a complex of four plants in intimate contact at the base. The rhizoidal filaments were interwoven somewhat, but could be separated with needles. Of the four, two were male, two female. The four spores from one tetrasporangium of *Griffithsia* frequently remain in contact after being shed, so that four spores may often be seen lying in immediate proximity, all derived from the same sporangium. In the cases mentioned, it seems as if this must have happened, and the four individuals composing the single compound plant have been derived from the four spores of a single sporangium. Further experiments will be made to settle this point.

V. *Dasya elegans*.—Carpospores could not be obtained early enough in the season to give positive results. Tetraspores sown July 18, 1911, transferred to Fay Wharf July 19, shells collected August 14. The stand of *Dasya* was quite good, but the individuals remained clearly separate, not running together at the base as in the case of *Griffithsia*. The largest specimens measured 4.5 cm. long, with 6 or 8 side branches from the main stem. The average length was 2 cm. Of the largest and best developed specimens, measuring more than 3 cm. in length, 6 were female, 7 male, and 1 sterile. Of the total number of individuals, 139 were sterile, 143 male, and 6 female. In interpreting this result, it is necessary to bear in mind that antheridia may develop when the plants are quite small (0.5 cm. long or in exceptional cases even less), while procarps do not begin to form in individuals less than about 3 cm. long. On control shells no *Dasya* developed. The most abundant contaminating species was *Polysiphonia variegata*, while *Champia parvula*, *Ceramium rubrum*, *Chondria tenuissima*, and *C. dasyphila*, along with *Enteromorpha* sp., occurred rarely.

What may be called the reciprocal cultivation of a single species has not yet been attended with success. It is more difficult to raise carposporelings to maturity than tetrasporelings, for the reason that tetrasporic plants are usually late in fruiting, while sexual individuals may be expected to produce reproductive bodies in 3–5 weeks. The experiments in their present status show, however, that in *Griffithsia* and *Dasya* the tetraspores actually do produce sexual plants, and only these, and that in *Polysiphonia violacea* carpospores produce only tetrasporic plants. The results of the experiments go to show, therefore, that the conclusions drawn from cytological evidence are valid, and that alternation of sexual and tetrasporic plants in the Florideae is now an observed fact.

In conclusion, it is a pleasure to acknowledge my indebtedness to the friends who have assisted me in various ways in the progress of this work, particularly Professor GEO. T. MOORE, Dr. ERNST A. BESSEY, Mr. H. WASTENEYS, and the officers of the Supply Department of the Marine Biological Laboratory.

Summary

1. There is no evidence that the double number of chromosomes in the carpospores imparts greater vigor of growth as compared with the single number of the tetraspores.

2. From the carpospores of *Polysiphonia violacea* 6 tetrasporic plants were obtained, and none sexual.

3. From the tetraspores of *Griffithsia Bornetiana* 60 sexual individuals were produced, and none tetrasporic.

4. From the tetraspores of *Dasya elegans* 149 sexual plants were secured, and none tetrasporic.

5. Tetraspores from a single individual produced male and female plants in approximately equal numbers in *Griffithsia*. The preponderance of males in *Dasya* is explained by the early fruiting of these as compared with the females.

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